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Green Synthesis of Silver and Zinc Nanoparticles and their Antimicrobial Activity

Introduction

Nanoparticles (NP) are inorganic particles less than 100 nm in size. Nanoparticles show unique physical, chemical and biological properties at nanoscale as compared to their properties at higher scales. They also differ in shape and size from the bulk material (Sadatzadeh &Charati,2018). Nanoparticles have large surface area to volume ratio because of that they can interact with other particles very easily (Agrawal, Kumar & Kumar, 2017). They are present in nature as well as synthesized by human beings. Nanoparticles are involved in various health, medicine, chemical and cosmetics industry of consumers (Sastry, Khan & Kumar, 2003). Nanoparticles works as catalysts, biosensors, conductors and are useful as diagnostic tools and cytotoxic and antimicrobial agents (Sharma, Rajgovind & Dut, 2017). Nanoparticles have significant antimicrobial property and are also considered as Nano-antibiotics (Agrawal, Kumar & Kumar, 2017).

The popularity of green biosynthesis of nanoparticles has increased in recent years and due to this lots of research have been conducted on synthesis of nanoparticles using different sources like bacteria, fungi, algae and plants. The most preferred source for NPs synthesis are plants because they are suitable for large-scale production and produce stable NPs as well as NPs of different shape and size (Qu, Yuan, Wang & Shao, 2011). Synthesis of NPs using natural extracts of plant parts is comparatively, cheap, eco-friendly and rapid and it does not involve usage of any intermediate base groups. It does not involve usage of costly equipments and precursors and gives a highly pure and quantity enriched product that is free from impurities (Kumar, 2018).

S. no.	Plants	Plants Tissue for extraction	Types of nanoparticles	Applications	References
1	*Euphorbia prostrata*	leaves	Silver and titanium oxide nanoparticles	Leishmanicidal	(Zahir, 2015)
2	*Ginkgo biloba, citrus plant*	Leaves	Copper	Catalytic, Antimicrobial Activity.	(Shende, Ingle,Gade, Rai, 2015)
3	*Azadirachta indica* *Pinus densiflora,* *Anogeissus latifolia*	Leaves, Seed, flower	Silver	Bactericidal	(Velmurugan, 2015) (Bhuyan, Mishra, Khanuja, Prasad, & Verma, 2015) (Kora, 2012)
4	*Catharanthus roseus*	leaves	Palladium	Catalytic activity in dye degradation.	(Kalaiselvi, 2015)

Table 1. Synthesis and application of nanoparticles from plants

Review of Literature

The characteristic properties of nanoparticles are affected by methods of nanoparticles synthesis, size and shape of nanoparticles and interactions with stabilizers (Vivehananthan, Sudha Lakshmi, De Silva & Kumar, 2015; Ingale & Chaudhari , 2013). Therefore, nanoparticles synthesis and maintain their stability is a big challenge for researcher (Vivehananthan, Sudha Lakshmi, De Silva & Kumar, 2015). The metal NPs can be synthesized and stabilized by many physical, chemical, and biological methods; chemical methods such as electrochemical approach, chemical reduction, pyrolysis (Qiaoxin, Hao, Xiaohui W & Xiaoliang, 2009) and photochemical reduction (Ahmad, Mukherjee, Senapati, Mandal & Khan, 2003) (Jacob, Kapoor, Biswas & Mukherjee, 2007) (Sharma, Yngard & Lin, 2009) and many physical methods such as physical vapour condensation and arc discharge (Tavakoli, Sohrabi & Kargari, 2007) are used. However, many of these nanoparticle synthesis methods uses hazardous chemicals or high energy for synthesis of nanoparticles, which are either difficult or include wasteful purifications (Ahmed, Ahmad & Swami, 2016). Biological systems such as the use of plant materials give an eco-friendly and alternative source for the production of nanoparticles (Antony, Gunasekaran, Sathiavelu & Arunachalam, 2016). There are several advantages of biosynthesis of nanoparticles using plant sources such as cost-effectiveness, eco-friendliness, less energy requirements than traditional synthesis methods (Sun & Xia , 2002).

Silver (Ag) is reported as an efficient antimicrobial substance against various pathogens *in vitro* and *in vivo* (Simone, Gallo, Paladini & Sannino, 2014). Bacteria are less prone to develop resistance against Ag as compared to conventional antibiotics (Leid, Ditto & Knapp, 2012) (Epple & Chernousova, 2013). Silver nanoparticles also works as an efficient inorganic catalyst (Lalitha & Firdhouse, 2015). Zinc nanoparticles have various biomedical applications, such as a drug delivery, anticancer, antibacterial, anti-inflammation wound healing and bio imaging (Jinhuan & Cai, 2018). Zinc nanoparticles have shown antimicrobial property against urinary tract pathogens (Santhosh, Venkat & Rajeshkumar, 2017) and against biofilm-producing methicillin-resistant Staphylococcus aureus (Jesline, John, Narayanan, Vani & Murugan, 2015). Antimicrobial property of Zinc nanoparticles was also reported against Gram Positive and Gram Negative bacteria (Bhuyan, Mishra, Khanuja,

Prasad & Verma, 2015). ZnO nanoparticles shown good antimicrobial activity against bacteria even at very low concentration. (Venkatachalam, Jayaraj, Manikandan, Geetha, 2016) (Hazra, Kundu, Chaudhari & Tushar, 2013).

For the synthesis of ZnO NPs plant parts like leaf, stem, root, seed, and fruit have been used. Synthesis of ZnO nanoparticles has been already reported from various plants, for example *Azadirachta indica* (Bhuyan, Mishra, Khanuja, Prasad & Verma, 2015), *Camella sinensis* (Shah, Boruah & Parween, 2015), *Cotton seed* (Sadatzadeh & Charati, 2018), *Bauhinia tomentosa* (Thirumarimurugan, Aswitha, Kiruthika, Nagarajan & Christy, 2018), *Scadoxus multiflorus* (Naif Abdullah Al-Dhabi & Valan, 2018), *Ocimum Tenuiflorum* (Raut, Thorat, & Thakre, 2013), *Aloe vera, Sargassum muticum* (Santhosh, Venkat & Rajeshkumar, 2017), *Rosa canina* (Saeed, Meysam, Baharkar & Morteza, 2016), *Moringa oleifoera* (Matinisea, Fukua, Kaviyarasua & Maye, 2017) , *Ocimum basilicum* (Hasna, Rajeshwari & Venckatesh, 2014).

Similarly, various plants have been reported those are enable to synthesize Ag nanoparticles for examples *Aloe vera* (Chandran, Chaudhary, Pasricha & Ahma, 2006), *Jatropha curcas* (Bar, Bhui, Sahoo, Sarkar, Pyne & Misra, 2009), *Acalypha indica* (Krishnaraj, Jagan, Rajasekar, Selvakumar, Kalaichelvan & Mohan, 2010), *Azadirachta indica* (Shankar, Rai, Ahmad & Sastry, 2004), *Emblica officinalis* (Ankamwar, Damle, Ahmad & Sastry, 2005), *Capsicum annuum* (Shikuo, Yuhua, Anjjan, Xuerong, Qiu, Li & Qingfeng, 2007), *Diospyros kaki* (Song & Kim, 2008), *Carica papaya* (Jain, Daima, Kachhwaha & Kothari, 2009), *Jatropha curcas, Solanum melongena, Datura metel, Citrus aurantium* (Rajasekharreddy, Rani & Sreedhar, 2010).

Rationale of the study

In recent years' microorganisms have shown antibiotic resistance mechanism and it is imposing serious problem to the health sector. The mode of action of nanoparticles is different because, it directly interacts with the bacterial cell wall and does not need to penetrate the cells. Therefore, most of the antibiotic resistance mechanisms seen are irrelevant. There is a hope that nanoparticles would be less prone to develop resistant bacteria than antibiotics (Beyth, Houri-Haddad, Domb, Khan & Hazan, 2015).

Plants are better source of nanoparticle synthesis because they act as natural capping agents and as well as free from toxic chemicals (Resmi, Sreejamol & Pillai, 2014). Plants extract contains various organic compounds such as terpenoids, these are surface active molecule and helps in stability of nanoparticles (Thirumurugan, Aswitha, Kiruthika, Nagarajan & Christy, 2016).

It has been reported that the antimicrobial property of silver is due to its ability to form free radicals which induce bacterial cell damage (Kim, Kuk, Yu, Kim, Park & Lee, 2007), (Danilczuk, Lund, Sadlo, Yamada & Michalik, 2006). Similarly, ZnO nanoparticles affect bacterial cell by disrupting their integrity and potential, and by inducting reactive oxygen species production (Beyth, Houri-Haddad, Domb, Khan & Hazan, 2015).

Although lots of research has been done on the synthesis of nanoparticles but research work for the application of nanoparticles in biomedical field is very less specially against pathogens. The present study focuses on applications of Ag and Zn nanoparticles as antimicrobial substance against pathogens.

Objective of the study

1. Screening of selective plant varieties (*Ocimum tenuiflorum, Catharanthus roseus, Camellia sinensis, Moringa oleifera)* and their extracts for Ag and Zn NP synthesis.
2. Optimization of process parameters for Ag and Zn NP synthesis
3. Characterization of Ag and Zn NPs.
4. Evaluation of antimicrobial activity of Ag and Zn NP against different septicemia cases/isolates.

Methodology

Study- Biosynthesis of silver and zinc nanoparticles, their characterization and to study their antimicrobial activity against different pathogens.

Design-

- Screening of selected Plants varieties for Nanoparticle Synthesis
- Plant Extract + Metal Precursor = Nanoparticle Synthesis
- Characterization of NPs
- Analysis of antimicrobial activity of synthesized NPs

Sample- Different plant varieties will be collected from nearby botanical gardens. Extracts of different plant parts will be prepared by following standard procedures. The pathogenic bacterial samples will be collected from any recognized Microbiological Disease Diagnostic Laboratory.

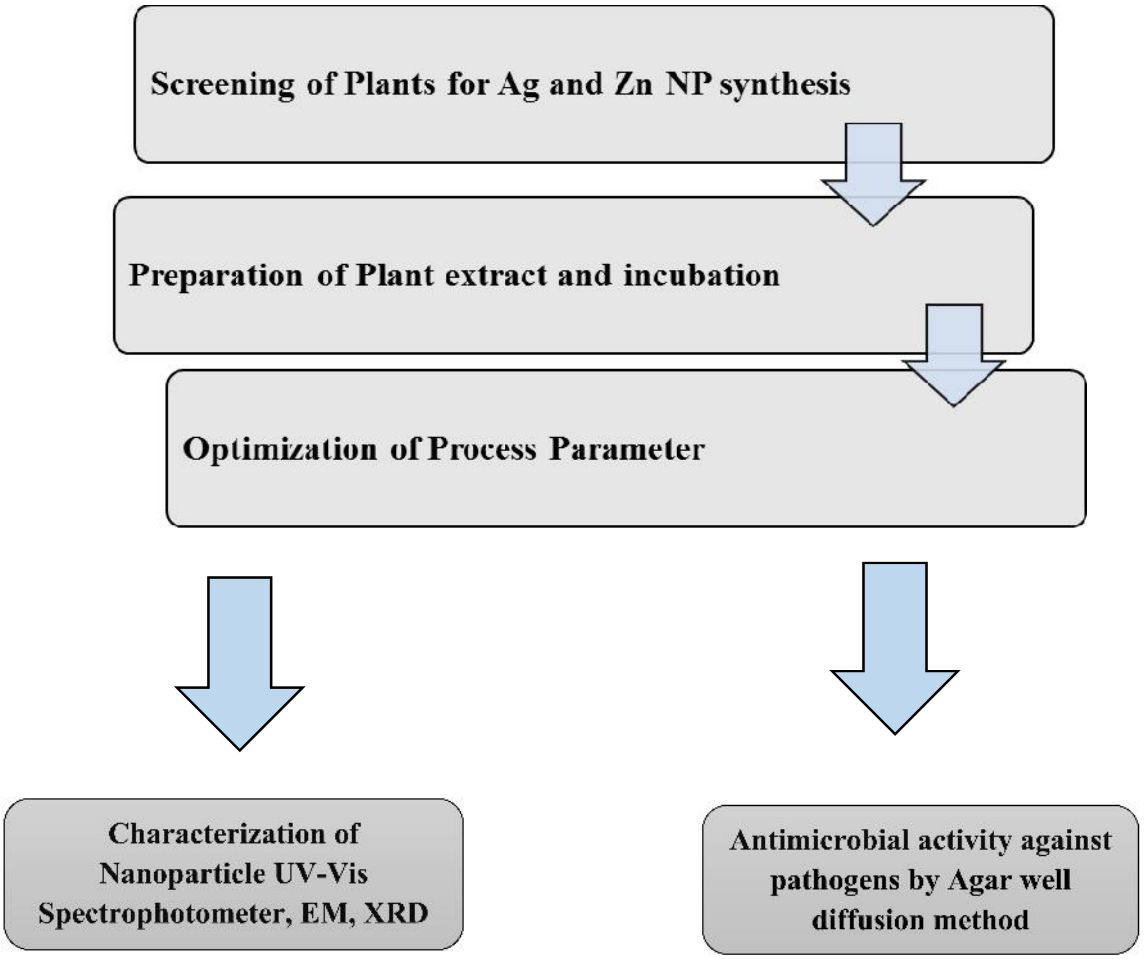
Tools for data collection- Different parameters affecting nanoparticle synthesis will be optimized for efficient nanoparticle synthesis. The efficiency of nanoparticles against different pathogens will be determined by agar well diffusion method and MIC value.

Tools for data analysis- The nanoparticles will be analysed using UV-Vis Spectrophotometry, Electron Microscopy, and XRD analysis.

UV-Vis Spectrophotometer analysis will be used for determining the absorbance of nanoparticles. Electron Microscopy studies will help in determining the size and morphology of nanoparticles and XRD analysis will be used in order to determine the crystallite size

Statistical Analysis- Complete data collected throughout the study will be analysed statistically for better presentation and understanding of results. Minor changes could be made in the plan if necessary or required.

Flow Chart of Methodology


Screening of Plants for Ag and Zn NP synthesis
Preparation of Plant extract and incubation
Optimization of Process Parameter
Characterization of Nanoparticle UV-Vis Spectrophotometer, EM, XRD
Antimicrobial activity against pathogens by Agar well diffusion method

Expected outcomes

The expected outcomes of the present study are-

1. Green synthesis of Ag and Zn Nanoparticles
2. Optimized parameters for Ag and Zn nanoparticle synthesis using selected plant extracts.
3. Size, shape and XRD details of the synthesized Ag and Zn nanoparticles.
4. Efficacy of the Ag and Zn nanoparticle against different septicimia isolates for commercial applications

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